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Enclosure: -Marked up draft of amended specification and claims

Marked up draft of amended specification and claims

In the specification:

(Page 3, line 12) Key components of the prokaryotic translation machinery have been identified in plastids, including homologues of the bacterial IF1, IF2 and IF3 initiation factors and an S1-like ribosomal protein (Stern et al., 1997). Most plastid mRNAs (92%) contain a ribosome binding site or SD sequence: GGAGG, or its truncated tri- or tetranucleotide variant. This sequence is similar to the bacterial SD consensus 5'-UAAGGAGGUGA-3' (SEQ ID NO: 28; Voorma, 1996). High level expression of foreign genes of interest in the plastids of higher plants is extremely desirable. The present invention provides novel genetic translational control elements for use in plastid transformation vectors. Incorporation of these elements into such vectors results in protein expression levels comparable to those observed for highly expressed chloroplast genes in both monocots and dicots.

(Page 4, line 15) In one embodiment of the invention recombinant DNA constructs for expressing at least one heterologous protein in the plastids of higher plants are provided. The constructs comprise a 5' regulatory region which includes a promoter element, a leader sequence and a downstream box element operably linked to a coding region of said at least one heterologous protein. The chimeric regulatory region acts to enhance translational efficiency of an mRNA molecule encoded by said DNA construct. Vectors comprising the DNA constructs are also contemplated in the present invention. Exemplary DNA constructs of the invention include the following chimeric regulatory regions: PrnnLatpB+DBwt, PrrnLatpB-DB, PrrnLatpB+DBm, PrrnLclpP+DBwt, PrrnclpP-DB, PrrnLrbcL+DBwt, PrrnLrbcL-DB, PrrnLrbcL+DBm,

PrrnLpsbB+DBwt, PrrnLpsbB-DB, PrrnLpsbA+DBwt, PrrnLpsbA-DB, PrrnLpsbA-DB(+GC), PrrnLT7g10+DB/Ec, PrrnLT7g10+DB/pt, and PrrnLT7g10-DB. Downstream box sequences preferred for use in the constructs of the invention have the following sequences: 5'TCCAGTCACTAGCCCTGCCTTCGGCA'3 (SEQ ID NO: 29) and 5'CCCAGTCATGAATCACAAAGTGGTAA'3 (SEQ ID NO: 30).

(Page 5, line 14) In yet another embodiment of the invention, at least one fusion protein is produced utilizing the DNA constructs of the invention. An exemplary fusion protein has a first and second coding region operably linked to the 5' regulatory regions described herein such that production of said fusion protein is regulated by said 5' regulatory region. In one embodiment the first coding region encodes a selectable marker gene and the second coding region encodes a fluorescent molecule to facilitate visualization of transformed plant cells. Vectors comprising a DNA construct encoding such a fusion protein are also within the scope of the present invention. An exemplary fusion protein consists an aadA coding region operably linked to a green fluorescent protein coding region. These moieties may be linked by peptide linkers such as ELVEGKLELVEGLKVA (SEQ ID NO: 104) and ELAVEGKLEVA (SEQ ID NO: 105).

(Page 9, line 26) **Figure 1A.** Plastid mRNAs and the small (16S) ribosomal RNA contain complementary sequences downstream of AUG implicating interactions between mRNA and 16S rRNA during translation initiation in plastids. Proposed model is based on data in *E. coli* (Sprengart *et al.*, 1996); for sequence of 16S rRNA (SEQ ID NO: 108) see ref. (Shinozaki *et al.*, 1986b). SD, Shine-Dalgarno sequence; ASD, anti SD region; DB, downstream box; ADB, anti DB region. Watson-Crick (line) and G-U (closed circle) pairing are marked.

(Page 10, line 2) **Figure 1B.** Sequence of the anti-downstream-box regions (ADB sequence underlined) of the 16S rRNA in plastids (pt; SEQ ID NO: 109; this application) and in *E. coli* (Ec; SEQ ID NO: 110; Sprengart et al., 1996). The *E. coli* ADB box contains sequences between nucleotides 1469-1483 of the 16S rRNA (Sprengart et al., 1996), corresponding to nucleotides 1416-1430 of the tobacco 16S rRNA (Dams et al., 1988; sequence between nucleotides 104173-104187 in Shinozaki et al., 1986).

(Page 10, line 12) **Figure 2A.** Base-pairing between plastid ADB (SEQ ID NO: 109) and wild type *atpB* (SEQ ID NO: 111), mutant *atpB* (SEQ ID NO: 112), *clpP* (SEQ ID NO: 113), wild type *rbcL* (SEQ ID NO: 114), mutant *rbcL* (SEQ ID NO: 115), *psbB* (SEQ ID NO: 116) and *psbA* (SEQ ID NO: 117) mRNAs (underlined). Multiple alternative DB-ADB interactions are shown. Nucleotides changed to reduce or alter mRNA-rRNA interaction are in lower case. The number of potential nucleotide pairs formed with the 26 nt ADB region is in parenthesis. The number of pairing events affected by mutagenesis is in bold.

(Page 10, line 20) **Figure 2B.** Complementarity of Prrn T7 phage gene 10 leader derivatives (T7g10, SEQ ID NO: 118; T7g10+DB/Ec, SEQ ID NO: 119; T7g10+DB/pt, SEQ ID NO: 120; T7g10-DB, SEQ ID NO: 121) with the *E. coli* (SEQ ID NO: 110) and plastid (SEQ ID NO: 109) ADB sequences. Nucleotides changed to reduce or alter mRNA-rRNA interaction are in lower case. The number of potential nucleotide pairs formed with the 26 nt ADB region is in parenthesis.

(Page 10, line 27) **Figure 3A.** DNA sequence of the chimeric Prrn plastid promoter fragments with *atpB* and *clpP* translation control regions (PrrnLatpB+DBwt, SEQ ID NO: 1;

PrrnLatpB-DB, SEQ ID NO: 2; PrrnLatpB+DBm, SEQ ID NO: 3; PrrnLclpP+DBwt, SEQ ID NO: 4; PrrnLclpP-DB, SEQ ID NO: 5). The plasmid name that is the source of the promoter fragment is given in parenthesis. The Prrn promoter sequence is underlined; nucleotide at which transcription initiates in tobacco plastids is marked with filled circle; translational initiation codon (ATG) is in bold; SD is underlined with a wavy line; nucleotides of the 5' and 3' restriction sites and point mutations are in lower case.

(Page 11, line 4) **Figure 3B**. DNA sequence of the chimeric Prrn plastid promoter fragments with *rbcL* and *psbB* translation control regions (PrrnLrbcL+DBwt, SEQ ID NO: 6; PrrnLrbcL-DB, SEQ ID NO: 7; PrrnLrbcL+DBm, SEQ ID NO: 8; PrrnLpsbB+DBwt, SEQ ID NO: 9; PrrnLpsbB-DB, SEQ ID NO: 10). For details see description of Fig. 3A.

(Page 11, line 8) **Figure 3C**. DNA sequence of the chimeric Prrn plastid promoter fragments with *psbA* translation control regions (PrrnLpsbA+DBwt, SEQ ID NO: 11; PrrnLpsbA-DB, SEQ ID NO: 12; PrrnLpsbA-DB(+GC), SEQ ID NO: 13). For details see description of Fig. 3A.

(Page 11, line 11) **Figure 3D**. DNA sequence of the chimeric Prrn plastid promoter fragments with the T7 phage gene 10 (PrrnLT7g10+DB/Ec; SEQ ID NO: 14) plastid (PrrnLT7g10+DB/pt; SEQ ID NO: 15) and synthetic DB (PrrnLT7g10-DB; SEQ ID NO: 16). For details see description of Fig. 3A.

(Page 12, line 28) **Figure 9.** DNA sequence of the Prrn(L)rbcL(S)::*neo*::TrbcL gene in plasmid pHK3 (SEQ ID NO: 17). Plasmid pHK2 carries an identical *neo* gene, except that there is an EcoRI site upstream of the SacI site.

(Page 13, line 22) **Figure 12**. Fraction of a codon encoding a particular amino acid and triplet frequency per 1000 codons in the mutagenized *atpB* and *rbcL* DB region (*atpB* wt: nucleotide sequence is nucleotides 1 through 42 of SEQ ID NO: 111, amino acid sequence is SEQ ID NO: 132; *atpB* m: nucleotide sequence is nucleotides 1 through 42 of SEQ ID NO: 112, amino acid sequence is SEQ ID NO: 132; *rbcL* wt: nucleotide sequence is nucleotides 1 through 42 of SEQ ID NO: 114, amino acid sequence is SEQ ID NO: 122; *rbcL* m: nucleotide sequence is nucleotides 1 through 42 of SEQ ID NO: 115, amino acid sequence is SEQ ID NO: 122; T7g10+DB/Ec: nucleotide sequence is SEQ ID NO: 123, amino acid sequence is SEQ ID NO: 124; T7g10+DB/pt: nucleotide sequence is SEQ ID NO: 125, amino acid sequence is SEQ ID NO: 126; T7g10-DB: nucleotide sequence is SEQ ID NO: 127, amino acid sequence is SEQ ID NO: 128). Altered nucleotides are in lower case.

(Page 16, line 19) **Figure 19.** The engineered bacterial *bar* coding region DNA sequence in plasmid pJEK3 and pJEK6 (SEQ ID NO: 18) and encoded amino acid sequence (SEQ ID NO: 129). Nucleotides encoding the *rbcL* five N-terminal amino acids are in lower case. Nucleotides added at the 3' end during construction are also in lower case. NcoI, BglII and XbaI cloning sites are marked.

(Page 16, line 27) **Figure 20A**. The synthetic *bar* gene DNA sequence (SEQ ID NO: 19) and the encoded amino acid sequence (SEQ ID NO: 130). The arginines encoded by AGA/AGG codons are in bold. Original nucleotides are in capital letters, altered bases are in lower case. Restriction sites used for cloning are marked.

(Page 16, line 32) **Figure 20B**. The synthetic *s2-bar* gene DNA sequence (SEQ ID NO: 20) and the encoded amino acid

sequence (SEQ ID NO: 130). The arginines encoded by AGA/AGG codons are in bold. Original nucleotides are in capital letters, altered bases are in lower case. Restriction sites used for cloning are marked.

(Page 17, line 24) **Figure 23A.** Plastid transformation vector with FLARE16-S as selectable marker targeting the plastid inverted repeat region. DNA (SEQ ID NO: 131) and protein (SEQ ID NO: 104) sequence at the *aadA-gfp* junction. Nucleotides derived from *aadA* and *gfp* are in capital, adapters sequences and the point mutation used to create the *Bst*XI restriction site (bold) are in lower case.

(Page 19, line 24) **Figure 28.** The sequence of FLARE16-S is shown (SEQ ID NO: 21).

(Page 19, line 26) **Figure 29.** The sequence of FLARE16-S1 is shown (SEQ ID NO: 22).

(Page 19, line 28) **Figure 30.** The sequence of FLARE16-S2 is shown (SEQ ID NO: 23).

(Page 19, line 30) **Figure 31.** The sequence of FLARE11-S is shown (SEQ ID NO: 24).

(Page 19, line 32) **Figure 32.** The sequence of FLARE11-S3 is shown (SEQ ID NO: 25).

(Page 20, line 1) **Figures 33A and 33B.** The sequence of pMSK35 is shown (SEQ ID NO: 26).

(Page 20, line 4) **Figures 34A and 34B.** The sequence of pMSK49 is shown (SEQ ID NO: 27).

(Page 32, line 6)

Table 2.

Oligonucleotides used for the construction of chimeric promoters.

#1: 5'-CCCGAGCTCGCTCCCCCGCCGTCGTTC-3' (SEQ ID NO: 31)

#2: 5'-CGAATTTAAAATAAATGTCCGCTTGCACGTCGATCGGTTAATTCTCCCAGAAATATAGCCATCC-3' (SEQ ID NO: 32)

#3: 5'-CCCGCTAGCCGTGGAAACCCCAGAACC-3' (SEQ ID NO: 33)

#4: 5'-CCCGCTAGCTCTCATAATAATAAAATAAATAAATATGTC-3' (SEQ ID NO: 34)

#5: 5'-TCACTTTGAGGTGGAAACGTAACTCCCAGAAATATAGCCATCC-3' (SEQ ID NO: 35)

#6: 5'-CCCGCTAGCTTCCTCTCCAGGACTTCG-3' (SEQ ID NO: 36)

#7: 5'-CCCGCTAGCAGGCATTAAATGAAAGAAAGAAC-3' (SEQ ID NO: 37)

#8: 5'-TAAGAATTTTCACAACAACAAGGTCTACTCGACTCCCAGAAATATAGCCATCC-3' (SEQ ID NO: 38)

#9: 5'-CCCGCTAGCTTTGAATCCAACACTTGCTTTAG-3' (SEQ ID NO: 39)

#10: 5'-CCCGCTAGCTGACATAAATCCCTCCCTAC-3' (SEQ ID NO: 40)

#11: 5'-CAAAGATAAATAGACACTACGTAACTTTATTGCATTGCTCCCAGAAATATAGCCATCC-3' (SEQ ID NO: 41)

#12: 5'-CCCGCTAGCATCATTCAATACAACGGTATGAACACG-3' (SEQ ID NO: 42)

#13: 5'-TTCTAGTGGGAAACCGTTGTGGTCTCCCTCCCAGAAATATAGCCATCC-3' (SEQ ID NO: 43)

#14: 5'-CCCGCTAGCCATATGTATATCTCCTTCTTAAAG-3' (SEQ ID NO: 44)

#15: 5'-CCCGCTAGCCTGTCCACCAGTCATGCTTGCCATA-3' (SEQ ID NO: 45)

#16: 5'-CCCGCTAGCCAAGGCAGGGCTAGTGATTGCCATATGTATATCTCCTTC-3' (SEQ ID NO: 46)

#17: 5'-TTTGTTTAACTTTAAGAAGGAGATATACATATGGCAAGCATGACTGGTGG-3' (SEQ ID NO: 47)

#18: 5'-CTCCTTCTTAAAGTTAAACAAAATTATTTCTAGTGGGAAACCGTTGT-3' (SEQ ID NO: 48)

#19: 5'-CAAAATAGAAAATGGAAGGCTTTTTGCTCCCAGAAATATAGCCATCCC-3' (SEQ ID NO: 49)

#20: 5'-CAAAATAGAAAATGGAAGGCTTTTTTCCCAGAAATATAGCCATCCC-3 (SEQ ID NO: 50)

#21: 5'-GGGCCATGGTAAAATCTTGGTTTATTTAATC-3' (SEQ ID NO: 51)

#22: 5'-GGGGCTAGCTCTCTCTAAAATTGCAGT-3' (SEQ ID NO: 52)

#23: 5'-GAATAGCCTCTCCACCCA-3' (SEQ ID NO: 53)

#24: 5'-CCCGCTAGCCGTGGACACCCCACTTCCACTTGTTGTCGGGTTTATTCTCAT-3' (SEQ ID NO: 54)

#25: 5'-CCCGCTAGCTTTGAATCCTACTGAGGCTTTTGTTTCTGTTTGAGGACTCAT-3'

(SEQ ID NO: 55)

(Page 42, line 7) RNA gel blot analysis was performed to determine steady-state levels of chimeric mRNA in the transplastomic lines. Total leaf RNA was prepared from the leaves and roots of plants grown in sterile culture according to Stiekema et al (1988). RNA (4 μg per lane) was electrophoresed on 1% agarose gel and transferred to nylon membranes using the PosiBlot Transfer apparatus (Stratagene). The blots were probed using Rapid Hybridization Buffer Amersham) with a ^{32}P-labeled *neo* probe (Pharmacia, Ready-To-Go Random Priming Kit). The *neo* probe was obtained by isolating the NheI/XbaI fragment from plasmid pHK2. The template for probing the tobacco cytoplasmic 25S rRNA was a fragment which was PCR amplified from total tobacco cellular DNA with primers 5'-TCACCTGCCGAATCAACTAGC-3' (SEQ ID NO: 56) and 5'-GACTTCCCTTGCCTACATTG-3' (SEQ ID NO: 57). RNA hybridization signals were quantified using a Molecular Dynamics PhosphorImager, and normalized to the 25S rRNA signal.

(Page 62, line 20) A NcoI/XbaI bar gene fragment was generated by PCR amplification using plasmid of pDM302 (Cao et al., 1992) with the following primers:

P1, 5'-AAACCATGGCACCACAAACAGAGAGCCCAGAACGACGCCC-3'
(SEQ ID NO: 58);

P2, 5'-AAAATCTAGATCATCAGATCTCGGTGACG-3' (SEQ ID NO: 59).

(Page 69, line 21)

Primer 1A ccATGgctAGCCCAGAAaGAaGaCCGGCCGAtATTaGaCG
(SEQ ID NO: 60)

Primer 1B GCATaTCaGCtTCtGTaGCACGtCtaATaTCGGCCGGtCt
(SEQ ID NO: 61)

Primer 2A TGCtACaGAaGCtGAtATGCCaGCaGTtTGtACaATCGTt
(SEQ ID NO: 62)

Primer 2B CTTGTtTCtATaTAaTGGTTaACGATtGTaCAaACtGCtG

(SEQ ID NO: 63)

Primer 3A AACCAtTAtATaGAaACAAGtACaGTaAACTTtaGaACtG

(SEQ ID NO: 64)

Primer 3B tTCtTGaGGTTCtTGaGGtTCaGTtCtaAAGTTtACtGTa

(SEQ ID NO: 65)

Primer 4A AaCCtCAaGAACCtCAaGAaTGGACtGAtGAtCTaGTCCG

(SEQ ID NO: 66)

Primer 4B AaGGATAGCGCTCtCGtAGACGGACtAGaTCaTCaGTCCA

(SEQ ID NO: 67)

Primer 5A TCTaCGaGAGCGCTATCCtTGGCTtGTaGCaGAaGTtGAC

(SEQ ID NO: 68)

Primer 5B GCGATaCCaGCtACtTCaCCGTCaACtTCtGCtACaAGCC

(SEQ ID NO: 69)

Primer 6A GGtGAaGTaGCtGGtATCGCaTAtGCGGGCCCtTGGAAGG

(SEQ ID NO: 70)

Primer 6B CCAaTCaTAtGCaTTtCtTGCCTTCCAaGGGCCCGCaTAt

(SEQ ID NO: 71)

Primer 7A CAaGaAAtGCaTAtGAtTGGACaGCtGAaTCaACtGTtTA

(SEQ ID NO: 72)

Primer 7B GtTGaTGaCGtGGtGAaACGTAaACaGTtGAtTCaGCtGT

(SEQ ID NO: 73)

Primer 8A CGTtTCaCCaCGtCAtCAaCGtACaGGACTtGGtTCtACt

(SEQ ID NO: 74)

Primer 8B TTCAGtAGaTGtGTaTAtAGaGTaGAaCCaAGtCCtGTaC

(SEQ ID NO: 75)

Primer 9A CTaTAtACaCAtCTaCTGAAaTCttTGGAGGCACAaGGtT

(SEQ ID NO: 76)

Primer 9B aACAGCtACaACaCTCTTaAAaCCtTGTGCCTCCAaaGAt

(SEQ ID NO: 77)

Primer10A TtAAGAGtGTtGTaGCTGTtATaGGatTGCCtAAtGAtCC

(SEQ ID NO: 78)

Primer10B CtTCaTGCATGCGtACaCtTGGaTCaTTaGGCAatCCtAT

(SEQ ID NO: 79)

Primer11A aAGtGTaCGCATGCAtGAaGCtCTaGGATATGCtCCaaGa

(SEQ ID NO: 80)

Primer11B CCtGCaGCCCtCAaCATaCCtCttGGaGCATATCCtAGaG
(SEQ ID NO: 81)

Primer12A GGtATGtTGaGGGCtGCaGGtTTCAAaCAtGGaAACTGGC
(SEQ ID NO: 82)

Primer12B tTGCCAaAAACCtACaTCATGCCAGTTtCCaTGtTTGAAa
(SEQ ID NO: 83)

Primer13A ATGAtGTaGGTTTtTGGCAaCTtGAtTTCAGtCTaCCaGT
(SEQ ID NO: 84)

Primer13B GtAGaACtGGACGaGGaGGTACtGGtAGaCTGAAaTCaAG
(SEQ ID NO: 85)

Primer14A ACCtCCtCGTCCaGTtCTaCCaGTtACtGAGATCTGATGA
(SEQ ID NO: 86)

Primer14B tctagaTCATCAGATCTCaGTaACtG
(SEQ ID NO: 87)

(Page 77, line 10) **Construction of tobacco plastid vectors.** The *aadA16gfp* gene encodes FLARE16-S fusion protein, and can be excised as an NheI-XbaI fragment from plasmid pMSK51, a pBSKSII+ derivative (Genbank Accesssion No. Not yet assigned . The fusion protein was obtained by cloning *gfp* (from plasmid pCD3-326F) downstream of *aadA* (in plasmid pMSK38), digesting the resulting plasmid with BstXI (at the 3' end of the *aadA* coding region) and NcoI (including the *gfp* translation initiation codon) and linking the two coding regions by a BstXI-NcoI compatible adapter. The adapter was obtained by annealing oligonucleotides 5'-GTGGGCAAAGAACTTGTTGAAGGAAAATTGGAGCTAGTAGAAGGTCTTAAAGTCGC-3' (SEQ ID NO: 88) and 5'-CATGGCGACTTTAAGACCTTCTACTAGCTCCAATTT TCCTTCAACAAGTTCTTTGCCCACTACC-3' (SEQ ID NO: 89). The adapter connects AAD and GFP with a peptide of 16 amino acid residues (ELVEGKLELVEGLKVA; SEQ ID NO: 104).

(Page 77, line 26) The engineered *aadA* gene (Chinault *et al.*, 1986) in plasmid pMSK38 (pBSIIKS+ derivative) has NcoI and NheI sites at the 5' end and BstXI

and XbaI sites at the 3' end of the gene. The NcoI site includes the translation initiation codon; the NheI and BstXI sites are in the coding region close to the 5' and 3' ends, respectively; the XbaI site is downstream of stop codon. The mutations were introduced by PCR using oligonucleotides 5'-GGCCATGGGGGCTAGCGAAGCGGTGATCGCCGAAGTATCG-3' (SEQ ID NO: 90) and 5'-CGAATTCTAGACATTATTTGCCCACTACCTTGGTGATCTC-3' (SEQ ID NO: 91).

(Page 78, line 4) The *gfp* gene in plasmid CD3-326F is the derivative of plasmid psmGFP, encoding the soluble modified version of GFP (accession number U70495) obtained under order number CD3-326 from the Arabidopsis Biological Resource Center, Columbus, OH (Davis and Vierstra, 1998). The *gfp* gene in plasmid CD3-326F is expressed in the PpsbA /TpsbA expression cassette. The *gfp* gene in plasmid CD3-326F was obtained through the following steps. The BamHI-SacI fragment from CD3-326 was cloned into pBSKS+ vector to yield plasmid CD3-326A. The SacI site downstream of the coding region was converted into an XbaI site by blunting and linker ligation (5'-GCTCTAGAGC; SEQ ID NO: 107; plasmid CD3-326B). An NcoI site was created to include the translation initiation codon and at the same time the internal NcoI site was removed by PCR amplification of the coding region N-terminus with primers 5'-CCGGATCCAAGGAGATATAACACCATGGCTAGTAAAGGAGAAGAACTTTTC-3' (SEQ ID NO: 92) and 5'-GTGTTGGCCAAGGAACAGGTAGTTTTCC-3' (SEQ ID NO: 93). The PCR-amplified fragment was digested with BamHI and MscI restriction enzymes, and the resulting fragment was used to replace the BamHI-MscI fragment in plasmid CD3-326B to yield plasmid CD3-326C. The *gfp* coding region was excised from plasmid CD3-326C as an NcoI-XbaI fragment and cloned into a *psbA* cassette to yield plasmid CD3-326D. PpsbA and TpsbA are the *psbA* gene promoter and 3'- untranslated region derived from plasmids pJS25 (Staub and Maliga, 1993). TpsbA has been truncated by inserting a HindIII linker downstream of the

modified BspHI site (Peter Hajdukiewcz, unpublished). The PpsbA::gfp::TpsbA gene was excised as an EcoRI-HindIII fragment and cloned into EcoRI and HindIII digested pPRV111A, to yield plasmid CD3-326F.

(Page 80, line 14) The *aadA* gene in plasmid pMSK49 was obtained by modifying the *aadA* gene in plasmid pMSK38 (above) to obtain plasmid pMSK39. The modification involved translationally fusing the *aadA* gene product at its N-terminus with an epitope of the human c-Myc protein (amino acids 410-419; EQKLISEEDL; SEQ ID NO: 106; Kolodziej and Young, 1991). The genetic engineering was performed by ligating an adapter obtained by annealing complementary oligonucleotides with appropriate overhangs into NcoI-NheI digested pMSK38 plasmid. The oligonucleotides were: 5'-CATGGGGGCTAGCGAACAAAAA CTCATTTCTGAAGAAGACTTGc-3' (SEQ ID NO: 94) and 5'-CTAGGCAAGTCTTCTTCAGAAATGAGTTTTTGTTCGCTAGCCCC-3' (SEQ ID NO: 95).

(Page 80, line 26) The *aadA11gfp* gene encoding FLARE11-S was obtained by linking AAD and GFP with the 11-mer peptide ELAVEGKLEVA (SEQ ID NO: 105). To clone *aadA* and *gfp* in the same polycloning site, *gfp* (EcoRI-HindIII fragment; from plasmid CD3-326F) was cloned downstream of *aadA* in plasmid pMSK39 to obtain plasmid pMSK41. The two genes were excised together as an NheI-HindIII fragment, and cloned into plasmid pMSK45 to replace a kanamycin-resistance gene yielding plasmid pMSK48. Plasmid pMSK45 is a derivative of plasmid pMSK35 which carries the PrrnLT7g10+DB/Ec promoter. The promoter consists of the plastid rRNA operon promoter and the leader sequence of the T7 phage gene 10 leader. In plasmid pMSK48, *aadA* is expressed from the PrrnLT7g10+DB/Ec promoter . The *aadA* and *gfp* genes were then translationally fused with an BstXI-NcoI adapter that links the AAD and GFP with an 11-mer peptide. The adapter was obtained by annealing oligonucleotides 5'-

GTGGGCAAAGAACTTGCAGTTGAAGGAAAATTGGAGGTCGC-3' (SEQ ID NO: 96) and 5'-CATGGCGACCTCCAATTTTCCTTCAACTGCAAGTTCTTTGCCCACTACC-3' (SEQ ID NO: 97), which was ligated into BstXI/NcoI digested pMSK48 plasmid DNA to yield plasmid pMSK49. Plasmid pMSK49 has the rice plastid targeting sequences present in plasmid pMSK35.

(Page 82, line 28) **PCR amplification of border fragments.** Total cellular DNA was extracted according to Mettler (Mettler, 1987). The PCR analysis was carried out with a 9:1 mixture of AmpliTaq (Stratagene) and Vent (New England Biolabs) DNA polymerases in the Vent buffer following the manufacturer's recommendations. The left border fragment was amplified with primers O3 (5'-ATGGATGAACTATACAAATAAG-3'; SEQ ID NO: 98) and O4 (5'-GCTCCTATAGTGTGACG-3'; SEQ ID NO: 99). The right border fragment was amplified with primers O5 (5'-ACTACCTCTGATAGTTGAGTCG-3'; SEQ ID NO: 100) and O6 (5'-AGAGGTTAATCGTACTCTGG-3'; SEQ ID NO: 101). The aadA part of FLARE-S genes was amplified with primers O1 (5'-GGCTCCGCAGTGGATGGCGGCCTG-3'; SEQ ID NO: 102) and O2 (5'-GGGCTGATACTGGGCCGGCAGG-3'; SEQ ID NO: 103). Primer positions are shown in Fig. 5A. Note that the same primers can be used in transplastomic tobacco and rice plants expressing FLARE-S.

(Page 84, line 22) Two FLARE-S fusion proteins were tested in *E. coli*. In one, the AAD and GFP were linked by an 11-mer (ELAVEGKLEVA; SEQ ID NO: 105), in the second by a 16-mer (ELVEGKLELVEGLKVA; SEQ ID NO: 104) linker. For transformation in tobacco, the *aadA16gfp* coding region (16-mer linker) was expressed in two cassettes known to mediate high levels of protein accumulation in plastids. Both utilize the strongest known plastid promoter driving the expression of the ribosomal RNA operon (Prrn), and the 3'-UTR of the highly expressed *psbA* gene (TpsbA) for the stabilization of the chimeric mRNAs. The PrrnLatpB+wtDB (plasmid pMSK56) and

PrrnLrbcL+DBwt (plasmid pMSK57) promoters utilize the *atpB* or *rbcL* gene leader sequences and the coding region N-termini with the downstream box (DB) sequence, respectively. Due to inclusion of the DB sequence in the chimeric genes, the proteins encoded by the two genes are slightly different, having 14 amino acids of the ATP-ase β subunit (*atpB* gene products) or ribulose 1,5-bisphosphate carboxylase/oxygenase (*rbcL* gene product) translationally fused with FLARE16-S (FLARE16-S1 and FLARE16-S2, respectively). To obtain a plastid transformation vector with the fluorescent spectinomycin resistance genes, the chimeric genes were cloned into the *trnV/rps12/7* plastid intergenic region in plastid vector pPRV111B. Plasmids pMSK56 and pMSK57 (Fig. 23) express FLARE16-S1 and FLARE16-S2, respectively, as markers.

In the claims:

6. A DNA construct as claimed in claim 1, said downstream box element having a sequence selected from the group consisting of 5'TCCAGTCACTAGCCCTGCCTTCGGCA'3 (SEQ ID NO: 29) and 5'CCCAGTCATGAATCACAAAGTGGTAA'3 (SEQ ID NO: 30).

13. A DNA construct as claimed in claim 10, said aadA coding region being operably linked to said green fluorescent protein coding region via a nucleic acid molecule encoding a peptide linker having a sequence selected from the group consisting of ELVEGKLELVEGLKVA (SEQ ID NO: 104) and ELAVEGKLEVA (SEQ ID NO: 105).